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Hematological and biochemical parameters in the indigenous Croatian white goat in relation to age

Zvonko Antunović[1], Ozren Erceg[2], Željka Klir Šalavardić[1*], Boro Mioč[3], Mislav Đidara[1], and Josip Novoselec[1]

[1]Department for Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, University of J. J. Strossmayer, Osijek, Croatia
[2]Croatian Agency for Agriculture and Food, Zagreb, Croatia
[3]Department of Animal Science and Technology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

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ABSTRACT

The aim of the research was to determine the age-related hematological and biochemical parameters in Croatian white goats in a traditional Mediterranean production system. The 36 lactating Croatian white goats were divided into three groups with 12 goats each, according to age: group I (1-2 years old), group II (3-5 years) and group III (>5 years). The mean corpuscular haemoglobin concentration (334 g/L) and lymphocytes (73.71%), as well as the concentration of iron (Fe 25.17 μmol/L) in the blood were higher, while the concentration of urea (3.86 mmol/L) was lower in group I compared to group III. The results of the present study are the first published blood analysis (hematological and biochemical parameters) for the Croatian white goat breed, and can be useful for understanding the metabolism and health status of this breed of goats, which helps in their conservation, promotion and breeding improvement.

Key words: age; Croatia; goats; blood parameters

Introduction

The knowledge of normal values of hematological and biochemical parameters of different small ruminants is of academic as well as of practical importance for clinical and experimental interpretations (ANTUNOVIĆ et al., 2013; ANTUNOVIĆ et al., 2017b). Therefore, determination of hematological and biochemical parameters is very important for monitoring the metabolism and health status of small ruminants (ANTUNOVIĆ et al., 2017b; MANAT et al., 2016; BROWN et al., 2016; ANTUNOVIĆ et al., 2017a). Blood components of goats (hematological and biochemical parameters) may be influenced by various factors, such as breed, age, reproductive status, sex, nutrition or season (ANTUNOVIĆ et al., 2017a; PAMBU-GOLLAH et al., 2000; PICCIONE et al., 2010; RUMOSA GWAZE et al., 2010; RUMOSA GWAZE et al., 2012; ARFUSO et al., 2016; ANTUNOVIĆ et al., 2020a). Red blood cells in goats´ blood are smaller in size compared

*Corresponding author:
Željka Klir Šalavardić, PhD assistant professor; Faculty of Agrobiotechnical Sciences Osijek, J. J. Strossmayer University of Osijek, V. Preloga 1, 31000 Osijek, Croatia, Phone: +385 31 554 906, E-mail: zklir@fazos.hr

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to those of other species, and when determined in automatic apparatus they are known to produce false values in goats (ARFUSO et al., 2016). Therefore, particular attention should be paid to their measurement. The biochemical parameters, primarily of minerals, metabolites and enzymes, of the blood indicate possible metabolic disorders, and disorders caused by inadequate nutrition (RIOS et al., 2006; ANTUNOVIĆ et al., 2019). Besides all these factors influencing the hematological and biochemical parameters of animals, climate zones and regions (locations) also affect these parameters. Therefore, it is important to specify the exact values for every breed of goats of all ages. These factors must be considered when establishing criteria for disease and physiological status.

Croatian white goats are an indigenous breed in Croatia. According to the CROATIAN AGENCY FOR AGRICULTURE AND FOOD (HAPIH, 2021), the estimated population of Croatian white goats is 5,000 head, and the breeding population is small and amounts to only 25 head in 11 breeders (246 head, 189 does, 41 female yearlings and 16 bucks). It is a combined breed (meat-milk) with the emphasis on the main product - goat meat. The goats have a white coat which is slightly longer than European white breeds of goats. It is assumed that it was created in the process of improving milk production through the breeding of domestic indigenous goats with imported foreign goats, most often the Sannen breed. The Croatian white goat is a resilient, adaptable, very mobile and lively goat. The average body weight is about 45 kg, height of withers 64 cm, body length 65 cm, shin circumference 8.6 cm, chest width and depth 16 and 28 cm, respectively (ANTUNOVIĆ et al., 2020b). The Croatian white goat, in lactation from 250 to 280 days, produces 250 - 350 l of milk (MIOČ and PAVIĆ, 2002). According to data of the HAPIH (2021), in the population of white goats under selection control, the index of kidding was 1.18, litter size 1.05, and birth weight 3.3 kg. A small number of Croatian white goats are under selection, which is a consequence of extensive rearing where monitoring of production is not possible. In the available literature, only one paper was found regarding production traits of Croatian white goats (ANTUNOVIĆ et al., 2020b) and there is no research related to the determination of hematological and biochemical parameters in the blood of this goat breed. Thus, the aim of this research was to determine the hematological and blood biochemical parameters of Croatian white goats in a Mediterranean production system, in relation to different ages of the goats.

Material and methods

Experimental design and animals. Analyses of hematological and biochemical parameters were made on 36 non-gravid Croatian white goats during the second half of lactation, of different ages in a traditional Mediterranean production system. These goats were selected from the herd of 50 animals. The selected goats were healthy, in good physical condition, and were divided into three groups of 12 goats each according to age, as follows; group I: 1-2 years old, group II: 3-5 years and group III: > 5 years. Goats grazed extensively on Mediterranean pastures starting in the early morning until 10 a.m., when they returned to the stall due to high temperatures, and were fed with hay (*ad libitum*) and 0.2 kg/day of corn on average. Water and salt were offered to the goats *ad libitum*. The present study was conducted in June 2019 when the goats were in late lactation (5th month of lactation). The family goat farm was located in Unešić, 25 km from Šibenik, Croatia.

Feeding and analyses. Corn, hay and green forage from pasture samples were dried and ground into a fine powder by using a heavy metal free ultra-centrifugal mill (Retsch ZM 200). Standard methods (AOAC, 2006) were used to determine feed composition, as presented in Table 1. In the feed samples, the crude protein content was determined by the Kjeldahl method and ether extract using a Universal Extractions System B-811 (Buchi, Switzerland). The Weende method was used to determine crude fiber content. The digestible and metabolizable energy of the feed was estimated according to DLG (1993). Digestion of feed samples was achieved with 10 ml of a 5:1 mixture of HNO_3 and H_2O_2 at 180°C (60 min) in a microwave oven (CEM Mars 6). Inductively

coupled plasma (ICP, PerkinElmer Optima 2100 DV) was used to determine the concentrations of mineral elements in solutions of digested plant samples. Each batch of plant samples run on the ICP was analyzed with an internal pooled plasma control, and with the reference material prepared in the same way as the other plant samples. All samples were analyzed in duplicate. The chemical compositions of the feed of the goats (mg/kg) are presented in Table 1.

Table 1. Chemical composition of ingredients in the goats´ diet

Parameters (% DM)	Feedstuffs		
	Green forage	Hay	Corn
Crude proteins	3.65	8.28	6.97
Crude fiber	25.98	31.47	2.16
Crude ash	0.38	5.02	1.28
Crude fat	0.97	1.31	3.72
Digestible energy, MJ kg^{-1} DM	4.24	8.26	15.79
Metabolizable energy, MJ kg^{-1} DM	4.11	7.53	13.45
Mineral content (mg/kg DM)			
Ca	79.48	10098.20	10150.46
Mg	1594.66	3086.07	3477.02
K	5755.42	6387.98	14922.26
P	4212.00	1162.38	2261.69
Na	68.03	1587.32	495.21
Fe	45.55	90.18	224.10

DM-dry matter

Blood collection and analysis. Blood samples were collected from each goat after the morning feeding, from the jugular vein (10 mL) into two sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium) containing ethylenediamine tetra-acetic acid (EDTA) as the anticoagulant, for hematology analysis. These tubes were inverted a few times to ensure proper mixing of the blood with the anticoagulant. Determination was made of hematological parameters (leukocyte-WBC and erythrocytes-RBC count, as well as the content of hemoglobin-HGB, hematocrit-HCT, mean corpuscular volume-MCV, mean corpuscular hemoglobin-MCH, and mean corpuscular hemoglobin concentration-MCHC) in the whole blood of the goats. Hematology analysis was carried out on an automated three part differential hematology analyser, Sysmex PocH-100iV (Sysmex Europe GmbH, Hamburg, Germany) intended for use in veterinary laboratories and veterinarian office laboratories. Blood smears were prepared, stained by Pappenheim, and a differential blood test was carried out by microscope. Blood samples collected in sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium) were centrifuged at 1609.92 *g* (10 min, at 4 ºC) and the serum samples obtained were set in Olympus AU400. In the serum, the concentration of minerals (calcium, phosphorus-inorganic, potassium, magnesium, iron, chloride), urea, glucose, total proteins, albumin, creatinine, cholesterol, triglycerides-TGC, bilirubin, β-hydroxybutyrate-BHB and non-esterified fatty acids-NEFA, as well as the activity of enzymes (alanine aminotransferase-ALT, aspartate aminotransferase-AST, alkaline phosphatase-ALP, creatine kinase-CK and γ-glutamyl transferase-GGT) were measured using Olympus System reagents (Olympus Diagnostic GmbH, Lismeehan, Ireland). Globulin content was calculated as the difference between total protein and albumin. The activity of glutathione peroxidase (GPx) in the serum was determined

using a Ransel® kit (Randox, UK) on an automatic Olympus AU 400 (Olympus, Japan) analyser at a wavelength of 340 nm. A Ransod® kit (Randox, UK) was used to determine the activity of total superoxide dismutase (SOD) in the serum with an automatic analyser (Olympus AU 400, Olympus, Japan) at a wavelength of 520 nm. The method is based on the generation of superoxide radicals from xanthine by xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4nitrofenil) 5-phenyltetrazole chloride to form formazan red coloration.

The present research was carried out under the legal provisions of the Animal Protection Act (Official Gazette no. 133 of 2006, No. 37 of 2013 and no. 125 of 2013) as confirmed by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences, Osijek. Animal care and the conditions of the research followed the recommendations of European Union Directive 86/609/EEC (1986).

Statistical analysis. The values for the haematological and biochemical parameters of goat's blood were performed by MEANS procedure. Analysis of variance was performed with a general linear model (GLM) procedure, with age as a fixed effect, while the differences between groups were determined using the Tukey test at a level of $P<0.05$ using SAS 9.4®.

Results

The analysis in Table 2 shows no significant differences ($P\geq0.11$) in hematological parameters, such as WBC, RBC, HGB, HCT, MCH and MCV, depending on the age of the Croatian white goats. Similarly, neutrophyls, eosinophyls, basophyls and monocytes did not differ significantly ($P\geq0.11$) between the different age groups of goats. Only MCHC content was significantly ($P<0.05$) higher in goats in group I (1-2 years) compared to group III (> 5 years) and the relative number of lymphocytes was significantly higher in group I compared to the other groups.

Table 2. Hematological parameters and leukocyte distribution in goats of different ages

	Group I (1-2 year)	Group II (3-5 years)	Group III (>5 years)	SEM	P-value	Reference values*
WBC ($\times10^9$ L)	9.82	10.65	8.75	0.40	0.11	4.0-13.0
RBC ($\times10^{12}$ L)	10.15	10.99	10.63	0.19	0.36	8.0-18.0
HGB (g/L)	79.17	78.91	77.20	1.75	0.28	80.0-120.0
HCT (L/L)	0.26	0.29	0.28	0.01	0.30	0.22-0.38
MCH (pg)	7.28	7.02	6.59	0.21	0.12	5.20-8.00
MCV (fL)	25.67	26.43	26.54	0.19	0.27	16.0-25.0
MCHC (g/L)	334.00^a	286.27^{ab}	279.30^b	9.97	0.02	300-360
Leukocyte distribution (%)						
Lymphocytes	73.71^a	61.92^b	63.55^b	1.62	0.03	50-70
Neutrophyls	19.57	23.75	24.50	1.46	0.48	30-48
Eosinophils	5.29	12.75	10.64	1.71	0.11	1-8
Basophils	1.29	1.50	1.23	0.18	0.81	0-1
Monocytes	0.14	0.08	0.08	0.04	0.70	0-4

*KRAMER (2000); SEM-pooled standard error of mean; a, b-values in rows with different letters differ significantly ($P<0.05$); WBC-number of leukocytes, RBC-erythrocytes, HGB-hemoglobin, HCT-hematocrit, MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemogobin concenteration.

In the blood of the Croatian white goats no significant (P≥0.12) changes were found between groups, regarding most of the biochemical parameters, such as Ca, P, K, Mg, glucose, total proteins, albumin, globulin, CHOL, TGC, BIL, NEFA and BHB, as presented in Table 3. In contrast, a significant (P<0.05) decrease was determined in the concentration of Fe and an increase in urea concentrations in group I (1-2 years) compared to group III (> 5 years).

Table 3. Biochemical parameters of goats of different ages

	Group I (1-2 year)	Group II (3-5 years)	Group III (>5 years)	SEM	P-value	Reference values*
Ca (mmol/L)	2.63	2.59	2.54	0.04	0.65	2.3-2.9
P (mmol/L)	2.25	2.17	2.05	0.09	0.59	1.0-2.4
K (mmol/L)	5.03	4.96	4.69	0.25	0.84	3.4-6.1
Mg (mmol/L)	1.19	1.22	1.19	0.02	0.59	0.8-1.3
Cl (mmol/L)	109.17	109.55	109.85	0.84	0.96	98.-110
Fe (µmol/L)	25.17[a]	21.94[ab]	18.96[b]	0.69	< 0.01	11.6-38.1[1]
Glucose (mmol/L)	1.50	1.65	1.82	0.09	0.08	2.4-4.0
Urea (mmol/L)	3.86[a]	4.47[ab]	5.21[b]	0.18	0.01	4-0-8.6
Total proteins (g/L)	75.22	76.92	78.87	1.15	0.50	62-79
Albumin (g/L)	31.55	31.05	29.30	0.44	0.80	29-43
Globulin (g/L)	43.67	45.87	49.57	1.14	0.12	35-57
CHOL (mmol/L)	2.13	2.02	1.96	0.06	0.64	1-3
TGC (mmol/L)	0.22	0.16	0.15	0.01	0.16	0.2
BIL (mmol/L)	5.00	4.64	5.80	0.73	0.78	0-7
NEFA (mmol/L)	0.10	0.10	0.09	0.00	0.13	<0.2[2]
BHB (mmol/L)	0.29	0.33	0.36	0.09	0.80	0-1.2

*JACKSON and COCKCROFT (2002); [1]KANEKO et al. (2008); [2]ANTUNOVIĆ et al. (2017a); SEM-pooled standard error of mean; a, b-values in rows with different letters differ significantly (P<0.05); CHOL-cholesterol, TGC-triglycerides, BIL-bilirubin; NEFA-non-esterified fatty acids, BHB-β-hydroxybutyrate.

Table 4. Activities of blood enzymes in serum from goats of different ages (U/L).

	Group I (1-2 year)	Group II (3-5 years)	Group III (>5 years)	SEM	P-value	Reference values
AST	109.15	103.90	109.80	3.35	0.74	46-161*
ALT	27.22	24.98	25.64	0.90	0.73	14-32[1]
ALP	356.14	230.75	185.22	54.47	0.43	200-255[2]
GGT	37.78	39.80	39.86	1.13	0.81	34-65*
CK	240.33	188.64	188.15	10.48	0.19	119-200[2]
SOD, U/mL	0.41	0.60	0.51	0.05	0.39	0.184[3]
GPx	221.67	138.74	185.49	1.14	0.20	> 600[4]

*SMITH (2002); [1]TSCHOUR et al. (2008); [2]ANTUNOVIĆ et al. (2017a); [3]MAAN et al. (2013); [4]PAVLATA et al. (2012); SEM-pooled standard error of mean; AST-aspartate aminotransferase, ALT-alanine aminotransferase, ALP-alkaline phosphatase, GGT-γ-glutamyl transferase, CK-creatine kinase, SOD-superoxide dismutase, GPx-glutathione peroxidise.

The activity of enzymes, such as AST, ALT, ALP, GGT and CK in the blood of Croatian white goats did not deviate significantly (P≥0.19) with increasing age. Similarly, the activities of enzymes in relation to antioxidant status did not differ significantly (P≥0.20) between goats of different ages, as presented in Table 4.

Discussion

No significant differences were found in relation to age in Croatian white goats, except for the content of MCHC, which was significantly higher in younger goats compared to older. Similarly, lymphocytes were significantly higher in the group of younger goats compared to the group of 3-5 year-old or even older goats. A lower MCHC content was found in goats older than 5 years compared to younger ones. In the study by EGBE-NWIYI et al. (2000) carried out on Nigerian goats, the authors also determined fluctations for MCHC values with increasing age, and also found higher levels of neutrophils with increasing age. On the contrary, ARFUSO et al. (2016) determined the lowest MCHC in young goats (1-2 years), increasing with age (>5 year) in different breeds from Italy. KRAMER et al. (2000) and HERMAN et al. (2018) reported a significant effect of age on bovine hematological parameters mainly concerning changes in young animals. In the blood of indigenous sheep in Ethiopia, a decrease in lymphocites was determined with increasing age in the study by TIBBO et al. (2005). Similar results for leukocyte distribution in Istrian goats was determined by ANTUNOVIĆ et al. (2020a). The deficiency of hemoglobin in the blood decreases the blood's oxygen-carrying capacity, leading to symptoms of anemia (AARON et al., 2003). However, anemia is a common abnormality seen in the blood profiles of ruminants defined as hematocrits less than 24%, a RBC count less than 5 $\times 10^{12}$ cells/L, or HGB concentration less than 8 g/dL (COLE et al., 1997). Therefore, in the present study there were no clear signs or symptoms of anemia in the goats. Increased numbers of basophiles may occur with some parasitic diseases (OTTER, 2013). Namely, an increase of neutrophiles could be a symptom of chronic inflammation and stress, or a sign of infections derived from the gastrointestinal tract (TORNQUIST and RIGAS, 2010).

No significant changes were found in concentrations of biochemical parameters in the blood of Croatian white goats with increasing age, except for concentrations of Fe and urea. With increasing age, there was a decrease in Fe concentrations and an increase in urea concentrations in all age categories of goats, and significant differences were found between groups I and III. Higher concentrations of Fe in the blood of Merinolanschaf ewes up to one year old could be connected to the rapid anabolic processes related to the increase in live body weight of ewes, as observed in the study by ANTUNOVIĆ et al. (2004), which could also be a reason for this in the present study.

The values of blood urea for adult sheep are higher than in young sheep or lambs (NJIDDA et al., 2014). The increase in the blood urea of goats with advancement of age could be a result of the greather efficiency of converting non-protein nitrogenous substances to amino acids and proteins. Similar results in rams in an arid tropical environment were determined by GATTANI and SAREEN (2011). Similarly, in the blood of Istrian goats, ANTUNOVIĆ et al. (2020a), determined a significant decrease in Fe and an increase in urea concentrations with advanced age. In the present study, a decrease in TGC and CHOL concentrations was observed with increasing age, but the differences depending on age were not significant. Similar results were found in goats´ blood in Iran by NAZIFI et al. (2002). The variations of mineral concentrations in the blood of Croatia white goats lie within the normal reference ranges for goats (JACKSON and COCKCROFT, 2002). The concentrations of glucose and NEFA, as well as BHB in the blood of goats are very good indicators of energy supply in goats (ANTUNOVIĆ et al., 2020a), while concentrations of urea are an indicator of protein supply (KOHN et al., 2005). VAN SAUN (2000) reported that BHB concentrations may not be sufficiently sensitive and may come from dietary sources. FERNANDEZ et al. (2007) reported that plasma NEFA indicates the energy status of goats. Blood NEFA concentration is linked to energy balance and 0.20-0.21 mmol/L has been suggested for lactating goats as zero energy balance (DUNSHEA and BELL, 1989). Blood glucose in domestic animals is regulated by the hypoglycaemic and hyperglycaemic hormones, but may be related to genetic predisposition (MERT et al., 2003). KARAPEHLIVAN et al. (2007) and BORJESSON et al. (2000) also determined lower glucose concentrations in sheep at the end of the

lactation period and during the dry period. Similar results for glucose concentrations in the blood of goats on Delftzyl farm were determined by PAMBU-GOLLAH et al. (2000). Similar results were also found by DURAK et al. (2015), suggesting that genetic adaptation may have developed for glucose. In dairy goats browsing Mediterranean shrubland LANDAU et al. (1993) found lower blood glucose concentrations, which were associtated with lower levels of concentrate supplementation and decreased milk production. KIRAN et al. (2012) did not determine any differences depending on age in the blood of goats in Pakistan in terms of concentrations of glucose, cholesterol, HGB, and activities of lactate dehydrogenase, AST and ALT, in a comparison of young goats (less than 12 month) and adults (more than 12 months).

Enzyme activity in the blood of Croatian white goats did not differ significantly with increasing age (Table 4). A comparison with the values reported by PAVLATA et al. (2012) for goats shows that they were within physiological limits, except for GPx concentrations which were significantly lower, and SOD which was higher. This indicates a possible lack of selenium in the food (pasture) of the goats, given the possibility of a lack of this trace element in the soil in the area. Significant soil areas in Southeast Europe, including Croatia, are poor in selenium (ANTUNOVIĆ et al., 2010, POPIJAČ and PRPIĆ-MAJIĆ, 2002). Increased soil acidity and the amount of selenium present in the soil not being available to plants may also lead to its deficiency in the diets of animals grazing on that soil. In the available literature we did not find any research related to selenium content in soil and animal diets at the location of the present study. Therefore, it is necessary to organize and conduct such research in the future, for better conclusions regarding the selenium supply in the soil. In the present study, a decrease in ALP activity was observed with the increasing age of the goats, but the differences were not significant. RUMOSA-GWAZE et al. (2012), in the blood of Nguni goats in South Africa, determined significantly higher levels of ALP in young (<1 year old) goats compared to mature goats. Similar findings in Girgetana goats in Italy were observed by PICCIONE et al. (2010) and in Croatian spotted goats by ANTUNOVIĆ et al. (2019). These changes migh be associated with the process of calcification (TOBA et al., 1992), which accompanies the growth and development of younger animals (ANTUNOVIĆ et al., 2004).

Conclusion

The results of the present study are the first published blood analysis (hematological and biochemical paramaters) of Croatian white goats, and thus can be useful for monitoring the metabolism and health status of these goats. This will help in the conservation, promotion and breeding improvement of this native Croatian breed. It was determined that goats older than 5 years had higher blood urea concentrations and lower Fe, MCHC and lymphocytes in comparison with goats aged 1 and 2 years. Therefore, in setting the reference values for Croatian white goats in the future, it is necessary to include age as an important factor, especially in determination of the concentrations of urea, Fe, glucose and the content of MCHC and lymphocytes.

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Conflict of interest statement
The authors declare that they have no conflict of interest

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SAŽETAK

Cilj istraživanja bio je utvrditi hematološke i biokemijske pokazatelje koza različite dobi, pasmine hrvatska bijela koza. Koze su uzgajane u tradicionalnom mediteranskom proizvodnom sustavu. Ukupno 36 koza u laktaciji podijeljeno je u tri skupine, svaka s po 12 koza, prema dobi: skupina I (dob 1-2 godine), skupina II (dob 3-5 godina) i skupina III (dob > 5 godina). Prosječna koncentracija hemoglobina u eritrocitima (334 g/l) i udio limfocita (73,71%), kao i koncentracija željeza (Fe 25,17 μmol/L) u krvi bile su više, dok je koncentracija uree (3,86 mmol/l) bila niža u skupini I u usporedbi sa skupinom III. Rezultati ovog istraživanja su prva objavljena analiza krvi (hematološki i biokemijski pokazatelji) za hrvatsku bijelu kozu. Utvrđene vrijednosti mogu biti korisne za razumijevanje metabolizma i zdravstvenog statusa ove pasmine koza, što pomaže u njezinom očuvanju, promociji i poboljšanju uzgoja.

Ključne riječi: dob; Hrvatska; koze; pokazatelji u krvi